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Microbiological analysis of tracheostomy tube biofilms and antibiotic resistance profiles of potentially pathogenic microorganisms

Authors' Contribution:
A – Study Design
B – Data Collection
C – Statistical Analysis
D – Data Interpretation
E – Manuscript Preparation
F – Literature Search
G – Funds Collection

Łukasz Ścibik[1,2,3ABCEF], Dorota Ochońska[1DEF], Monika Gołda-Cępa[2A], Andrzej Kotarba[2A], Monika Brzychczy-Włoch[1ADE]

[1]Department of Molecular Medical Microbiology, Chair of Microbiology, Faculty of Medicine, Jagiellonian University Medical College, Krakow, Poland; Head: prof. Monika Brzychczy-Włoch MD PhD
[2]Faculty of Chemistry, Jagiellonian University, Krakow, Poland
[3]Department of Otolaryngology and Oncological Surgery of the Head and Neck, 5th Military Hospital with Polyclinic in Krakow, Krakow, Poland; Head: col. Paweł Dobosz MD PhD

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ABSTRACT:

Introduction: In hospitalized patients, tracheostomy tubes (TTs) are susceptible to colonization by biofilm-producing potentially pathogenic microorganisms (PPMs). Contact with TTs, which are situated in a critical region of the body with enormous microbial exposure, may lead to the emer-gence of resistant respiratory infections.

Objective: Our study aimed to isolate and identify Gram-positive and Gram-negative PPMs, mark their antibiotic resistance and determine the bacteriological pattern of the biofilm colonizing the TTs.

Methods: The study was conducted on 45 tracheostomy tubes obtained from 45 hospitalized adult patients with tracheostomy with intubation periods ranging from 1 to 28 days. Tracheal aspirates (TA) obtained from polyvinyl chloride (PVC) TTs were used for the analysis. Bacteria in biofilms were identified by standard microbiological techniques, tested for antibiotic resistance and phenotypic resistance according to the EUCAST guidelines and visualized by SEM.

Results: Out of 45 TTs, 100% were found to be positive in bacterial cultures with 58 PPM isolates (10 spe-cies) correlating well with the SEM findings. Overall, 72% of isolates were Gram-negative bacilli, followed by Gram-positive cocci (28%). *Staphylococcus aureus* was the predominant bacterium (identified in 35.5% of patients), followed by *Klebsiella pneumoniae* (identified in 23.8%). Among the Gram-negative PPMs, 50% of isolates were identified as multidrug-resistant (MDR), 8.6% as extremely drug-resistant (XDR) and 5.2% were pandrug-resistant (PDR).

Conclusions: Our study showed a rapid colonization of the TT surface by biofilm- producing PPMs. Patients with tracheosto-mies, also those with non-infectious conditions, were mainly colonized with highly re-sistant bacteria.

KEYWORDS: antibiotic resistance, biofilm, potentially pathogenic microorganisms, tracheostomy tube

ABBREVIATIONS

AMC – Amoxicillin-clavulanic acid
BAIs – Biomaterial-Associated Infections
CAZ – ceftazidime
CIP – ciprofloxacin
CN – gentamycin
COPD – chronic obstructive pulmonary disease
cPPMs – community potentially pathogenic microorganisms
CRO – ceftriaxone
CRTH – radical chemoradiotherapy
CT – colistin
CXM – cefuroxime
DA – clindamycin
DDST – double-disc synergy tests
DRI – device-related infections
ENT – Ear, Nose and Throat
ESβL – extended-spectrum β-lactamase
EUCAST – European Committee on Antimicrobial Susceptibility Testing
hPPMs – hospital potentially pathogenic microorganisms
ICU – intensive care unit
IMP – imipenem
LEV – levofloxacin
MDR – multidrug-resistant
MEM – meropenem
MHA – Mueller-Hinton agar
MIC – Minimum Inhibitory Concentration
MLSB – macrolide, lincosamide and streptogramin B
MRSA – methicillin-resistant *S. aureus*
MSA – mannitol salt agar
MSSA – methicillin-sensitive *Staphylococcus aureus*
MTZ – metronidazole
NFGNB – non-fermenting Gram-negative bacteria
NR – natural resistance

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ORL – Department of Otolaryngology and Oncological Surgery of the Head and Neck
PDR – pandrug-resistant
PPMs – potentially pathogenic microorganisms
PVC – polyvinyl chloride
RTH – radiotherapy
SAM – ampicillin-sulbactam
SEM – scanning electron microscopy
TA – tracheal aspirates
TIA – transient ischemic attack
TSB – Tryptic Soy Broth
TTs – tracheostomy tubes
TZP – piperacillin-tazobactam
VA – vancomycin
VAP – ventilator-associated pneumonia
XDR – extensively drug-resistant

INTRODUCTION

Tracheostomy is one of the most common life-saving procedures that has been performed for many years in adults and children. Especially now, during the pandemic of covid-19, we are facing unique challenges for tracheostomy care [1, 2]. This operative method of unblocking the airway involves cutting the front wall of the trachea to form an opening called a tracheostomy, and then inserting a tracheostomy tube to keep the airway open and allow gas exchange [3]. Timing is a key criterion for performing tracheotomy (many clinicians use a specific time window) and patients who receive it, require a large amount of health care resources; however, proactive planning can optimize patient care. The use of the tracheotomy procedure has recently increased significantly [3].

In Poland, the percentage of patients admitted to intensive care units and requiring replacement ventilation is 74%, of which approximately 41% require tracheostomy due to prolonged ventilation [4]. In recent years, due to the growing number of patients operated on for head and neck cancers, and hence requiring tracheostomy, the number of these procedures performed in Ear, Nose and Throat (ENT) departments has increased [5].

The tracheostomy technique can be done with indications for emergency or planned surgery [6]. Planned tracheostomy is currently a commonly performed surgical intervention in critically ill patients in intensive care units, where it is inextricably linked with the performance of mechanical ventilation [7–10]. Although tracheotomy is described as a safe method, it is not devoid of early and late complications [6]. Bleeding may appear intraoperatively as well as difficulties in tracheal intubation and even retention circulation. As for early postoperative complications, the following are classified: early bleeding, periostomal infections, pneumothorax and displacement or a prolapsed tracheostomy tube. Early bleeding is caused by improper vascular supply during the procedure [6]. Other early complications also include periostomal infections, pneumothorax, incorrect site of tracheostomy tube insertion [9]. Late complications include late bleeding, development of a tracheo-esophageal fistula, strictures of the larynx and trachea [6].

Patients with tracheostomies are at particularly high risk for microbial colonization and subsequent pneumonia because of disrupted local clearance mechanisms, underlying immunosupresion, the frequency of invasive procedures, the wide use of respiratory therapy equipment and location in an intensive care environment with exposure to numerous nosocomial pathogens [7]. Moreover, the commonly used TTs are significant in patient care as they are situated in a critical region of the body subject to enormous microbial exposure, which may lead to the emergence of resistant device-related infections (DRI) and could be the source of serious TT-associated respiratory infections including ventilator-associated pneumonia (VAP). An episode of VAP may be due to a single pathogenic microorganism or have polymicrobial origin [9].

The bacterial etiology of VAP is highly varied and distinct patterns have been identified according to the duration of intubation. Early-onset VAP, developing within the first 2 to 5 days after intubation, has a better prognosis and is more likely caused by antibiotic-sensitive pathogens such as methicillin-sensitive *Staphylococcus aureus* (MSSA). Later occurring VAP, developing 5 or more days after the start of mechanical ventilation, has been associated with a higher morbidity and mortality and frequently involves antibiotic-resistant pathogens like methicillin-resistant *S. aureus* (MRSA), extended-spectrum β-lactamase (ESβL) producing *Enterobacteriaceae* and *Pseudomonas aeruginosa* [11]. The variety of microbiological factors associated with the pathogenesis of VAP makes it extremely difficult to develop effective preventive strategies to counteract them [7, 12, 13]. It was proved that regular screening of tracheal aspirates (TA) facilitates early identification of the microorganisms linked to VAP and has been shown to have impact on patient treatment and survival [9–10, 14].

One of the main mechanisms of TT colonization by potentially pathogenic microorganisms (PPMs) is biofilm formation. Biofilms are present on more than 90% of TTs within 7 days of insertion, and standard cleaning methods do not completely remove the bacteria [12]. Biofilms are associated with an increased risk of upper respiratory infections, facilitate bacterial contamination of the lower respiratory tract, TT occlusion and wound infections, among other complications. On the other hand, in intubated patients, the clinical distinction between bacterial colonization and pulmonary infection is often difficult to assess [14]. Bacteriological examination of TA is often misleading, due to the formation of these biofilms. Therefore, characterizing biofilms and identifying the bacterial species residing on the surface of TTs are of major importance.

AIM

Due to the above reasons, the aim of our study was to isolate and identify the most common Gram-positive and Gram-negative PPMs with determination of bacteriological profiles of the biofilm colonizing TTs and mark their related antibiotic resistance. In addition, bacterial biofilm structures on the surface of TTs were visualized by scanning electron microscopy (SEM).

Tab. I. Clinical characteristics of civil patients of the 5th Polish Military Hospital with Polyclinic in Krakow (Poland) on admission to the ORL (n = 35) and ICU (n = 10).

PATIENT NO. TTS ID	AGE/SEX	WARD	REASON FOR INTUBATION	DURATION OF INTUBATION	COMORBIDITIES	ANTIBIOTICS AT ICU, ORL ADMISSION	SMOKING (PER DAY)	CRTH/RTH
TT_1	72/M	ORL	Larynx cancer	1 d	Atherosclerosis, hypertension, hypercholesterolemia	AMC	20	–
TT_2	65/M	ORL	Larynx cancer	1 d		AMC	15	
TT_4	62/M	ORL	Larynx cancer	1 d	Diabetes, bronchiectasis	AMC	20	CRTH
TT_5	74/M	ORL	Larynx cancer	1 d	Hypertension, hypercholesterolemia	AMC		
TT_6	66/M	ORL	Larynx cancer	1 d	Hypothyroidism, thrombocytopenia	AMC	20	RTH
TT_7	81/K	ICU	Respiratory failure	3 d	Diabetes, hypertension	AMC, CN, TZP		
TT_8	89/K	ICU	Respiratory failure	6 d		SAM, CIP, CN, MEM		
TT_9	61/K	ORL	Tonsil cancer	1 d		AMC		RTH
TT_10	36/M	ICU	Respiratory failure	10 d		SAM, CAZ, CIP, CT, VA		
TT_11	87/K	ICU	Respiratory failure	12 d	Diabetes, hypertension, ischemic cardiac disease	SAM, DA, MTZ, TZP, VA		
TT_12	86/M	ORL	Larynx cancer	1 d		AMC		
TT_13	72/M	ORL	Larynx cancer	1 d	Hypertension	AMC	4	
TT_14	87/M	ORL	Larynx cancer	1 d		AMC		
TT_15	68/M	ORL	Larynx cancer	1 d	Depression, hypertension	AMC	20	
TT_16	61/M	ORL	Larynx cancer	1 d		AMC	20	RTH
TT_17	75/M	ORL	Larynx cancer	1 d	Hypertension	AMC	20	
TT_18	56/M	ORL	Larynx cancer	1 d	Atrial fibrillation, cardiomyopathy	AMC		RTH
TT_19	81/M	ORL	Larynx cancer	1 d	Cardiac Ischemia, hypertension	AMC	20	
TT_20	55/M	ORL	Larynx cancer	1 d		AMC	20	
TT_21	71/M	ORL	Larynx cancer	1 d	Cardiac Ischemia, diabetes, hypertension, peptic ulcer disease	AMC		
TT_22	77/M	ORL	Larynx cancer	1 d		AMC		
TT_23	85/K	ICU	Respiratory failure	28 d	Atrial fibrillation, diabetes, hypertension, ischemic heart disease	CT, IMP, VA		
TT_24	50/M	ORL	Larynx cancer	1 d		AMC		
TT_25	67/M	ORL	Larynx cancer	1 d	Arrhythmia, diabetes, hypertension	AMC	40	
TT_26	49/M	ORL	Larynx cancer	1 d		AMC	30	

Tab. I. cd. Clinical characteristics of civil patients of the 5th Polish Military Hospital with Polyclinic in Krakow (Poland) on admission to the ORL (n = 35) and ICU (n = 10).

PATIENT NO. TTS ID	AGE/SEX	WARD	REASON FOR INTUBATION	DURATION OF INTUBATION	COMORBIDITIES	ANTIBIOTICS AT ICU, ORL ADMISSION	SMOKING (PER DAY)	CRTH/RTH
TT_27	71/M	ORL	Larynx cancer	1 d	Choroba niedokrwienna serca	AMC	10	
TT_28	69/M	ORL	Larynx cancer	1 d		AMC	15	
TT_29	69/K	ORL	Larynx cancer	1 d		AMC	20	CRTH
TT_30	64/M	ORL	Larynx cancer	1 d		AMC	20	
TT_31	84/M	ICU	Respiratory failure	14 d	Arrhythmia, atrial fibrillation, diabetes, hypertension, ischemic heart disease	CXM, CIP, MEM		
TT_32	85/K	ICU	Respiratory failure	12 d	Atrial fibrillation, atherosclerosis, hypertension, hypothyroidism, TIA, valve defect (mitral tricuspid)			
TT_33	62/K	ORL	Larynx cancer	1 d	Atherosclerosis, diabetes, hypothyreosis, ischemic heart disease		20	
TT_34	81/M	ORL	Larynx cancer	1 d	Hypertension	AMC		RTH
TT_35	57/M	ORL	Larynx cancer	1 d	Atrial fibrillation, cardiomyopathy, ischemic heart disease	AMC		
TT_36	65/M	ORL	Larynx cancer	1 d	Hypertension	AMC	40	
TT_37	62/M	ORL	Larynx cancer	1 d	Diabetes	AMC	20	
TT_38	69/K	ICU	Respiratory failure	11 d	Alcoholism, COPD, diabetes, ischemic heart disease, hypertension, liver damage, psychotic disorders	CRO, CXM, LEV	20	
TT_39	68/M	ORL	Larynx cancer	1 d	Hyperparathyroidism, hypertension, ischemic heart disease, renal failure			
TT_40	71/M	ORL	Larynx cancer	1 d	Atherosclerosis, hypertension, peptic ulcer disease	AMC		
TT_41	64/M	ORL	Larynx cancer	1 d	Atherosclerosis, epilepsy	AMC	40	RTH
TT_42	70/M	ORL	Larynx cancer	1 d		AMC	10	
TT_43	63/M	ORL	Larynx cancer	1 d	Ischemic heart disease	AMC	20	
TT_44	83/K	ICU	Respiratory failure	11 d	Atherosclerosis, atrial fibrillation, valve defect (mitral, aortal)			
TT_45	68/M	ICU	Respiratory failure	9 d	Atrial fibrillation, hypertension			

Abbreviations: COPD – chronic obstructive pulmonary disease, CRTH – radical chemoradiotherapy, ICU – intensive care unit, F – female, M – male, ORL – Department of Otolaryngology and Oncological Surgery of the Head and Neck, RTH – radiotherapy, TIA – transient ischemic attack, TTs – tracheostomy tubes, VAP – ventilator-associated pneumonia.
Antibiotic shortcuts: AMC – Amoxicillin-clavulanic acid, CN – gentamycin, CAZ – ceftazidime, CIP – ciprofloxacin, CRO – ceftriaxone, CT – colistin, CXM – cefuroxime, DA – clindamycin, IMP – imipenem, LEV – levofloxacin, MEM – meropenem, MTZ – metronidazole, SAM – ampicillin-sulbactam, TZP – piperacillin-tazobactam, VA – vancomycin.

MATERIALS AND METHODS

Ethics

The study was approved by the Bioethics Committee of the Jagiellonian University in Krakow, Poland (KBET No. 1072.6120.153.2019).

Study design and patient characteristics

The present work was conducted in a 28-bedded Department of Otolaryngology and Oncological Surgery of the Head and Neck (ORL) and an 11-bedded Intensive Care Unit (ICU). The study included 45 patients (35 males/10 females), aged 36–94 years (mean: 75), of whom 35 were admitted to the ORL and 10 were admitted to the ICU during the study period (from October 2018 to February 2020). Some clinical data of patients are presented in Tab. I. All patients underwent intubation or tracheostomy due to impaired respiratory or lung function, laryngeal cancer or tonsil cancer and were intubated for at least 1 day. TTs were collected upon extubation of patients. Each TT was placed in a sterile specimen bag (Biohazard) and immediately frozen at –80°C until processing for bacterial culture. From the central region of each tube, a 5-cm section was cut and transferred to 15 ml of Tryptic Soy Broth (TSB). The buffer was then heated to 37°C for 30 minutes. TA was removed from the tube by repeated cycles (3x) of vortexing. Each time, 1 ml of the resulting cell suspension was used for cultivation. In total, 45 clinical samples (TA) from ventilated patients were analyzed. All were investigated by culture-dependent techniques.

Microbiological Methods

Isolation and Identification Techniques

Microbial cultures were inoculated on different isolation media: Columbia LAB-AGAR+5%KB (BIOCORP, Poland), Chocolate LAB-AGAR™ (CH+PV) (BIOCORP, Poland), MacConkey LAB-AGAR™ (BIOCORP, Poland), mannitol salt agar (MSA) (Oxoid, UK) and Mueller-Hinton agar (MHA) (BD, USA) and incubated at 37°C for 24 h. Pure microbial cultures were obtained from all isolation media and stored at –80°C using Microbank™-Dry vials (Pro-Lab Diagnostics, Birkenhead, UK). Conventional microbiological analyses (colony morphology, Gram staining characters, microscopic images, biochemical profiles, catalase and oxidase tests) were performed on all isolates using standard procedures [15].

- **Gram-positive PPMs**. β-hemolytic, Gram-positive, catalase-positive cocci were tested for the presence of coagulase enzyme (IBSS BIOMED S.A., Poland). All staphylococci were inoculated on an API® Staph strip (bioMérieux, France) according to the manufacturer's instructions, to confirm the species *S. aureus*. On the other hand, β-hemolytic, Gram-positive, catalase-negative and oxidase-negative (BD BBL™ DrySlide™, USA) streptococci were identified by API 20 Strep (bioMérieux, France) and the automated miniAPI system (bioMérieux, France). Lancefield grouping was conducted on cards using the rapid latex agglutination test: The Oxoid Dryspot Streptococcal Grouping Kit (Oxoid Ltd., UK). *Streptococcus faecalis* of group D (GDS, n = 1) was correctly grouped by using these reagents.
- **Gram-negative PPMs**. Gram-negative, oxidase-negative bacilli were identified by API 20E (bioMérieux, France). Isolates producing a greenish pigment were considered to be *Pseudomonas* species. These strains were inoculated on Mueller Hinton 2 LAB-AGAR™ (BIOCORP, Poland) plates that were incubated overnight at 37°C. The initial identification was confirmed by analyzing a selection of isolates by API 20 NE (bioMérieux, France). The remaining oxidase-positive, non-fermenting Gram-negative bacteria (NFGNB) were identified by API 20 NE (bioMérieux, France) and the automated miniAPI system (bioMérieux, France).
- **Potentially pathogenic microorganisms.** Microorganisms recognized as causing respiratory infections are referred to as potentially pathogenic microorganisms (PPMs). Additionally, PPMs were classified into predominantly community (cPPMs) or hospital (hPPMs) and non-PPMs, according to the definitions proposed by Drakulovic et al. followed by further researchers [8]. On the basis of these criteria, commensal flora (coagulase-negative *Staphylococcus* spp., *Corynebacterium* spp., *Enterococcus* spp., *Neisseria* spp., *Micrococcus* spp., *Moraxella catarrhalis*, Propionibacterium acnes and viridans group streptococci) were classified as non-PPMs and were excluded from antibiotic resistance studies. Furthermore, the above study did not include anaerobic bacteria or fungi.
- **Antibiotic Resistance.** Antibiotic resistance tests of the isolated PPMs were performed by the E-test method using the Liofilchem® MIC Test Strips (Liofilchem, Italy) to determine the Minimum Inhibitory Concentration (MIC) of 31 antibiotics as per the European Committee on Antimicrobial Susceptibility Testing (EUCAST, v. 10.0, 2020) guidelines [15]. Defining isolates as multidrug-resistant (MDR), extensively drug-resistant (XDR) and pandrug-resistant (PDR) was done according to the standardized international document [16].

Phenotypic screening of antibiotic resistance mechanisms among Gram-positive PPMs

- **Detection of methicillin resistance.** Methicillin resistance in the *S. aureus* isolates was checked by the cefoxitin disc (30 μg).
- **Testing for macrolide, lincosamide and streptogramin B (MLSB) resistance**. Detection of the macrolide, lincosamide and streptogramin B (MLSB) resistance mechanism was performed using the disc diffusion method with erythromycin (15 μg) and clindamycin (2 μg) discs (Oxoid, UK) and according to the EUCAST guidelines.

Phenotypic screening of antibiotic resistance mechanisms among Gram-negative PPMs

- **Screening of ESβL, AmpC and carbapenemase producers.** Double-disc synergy tests (DDST) were carried out to confirm ESβL production. Hodge and imipenem-EDTA (EURX, Poland) double-disc synergy tests were used to screen for MBL production. The detection of KPC was assessed by the disc test with phenylboronic acid. The detection of OXA-48 was done as previously published [17]. Temocillin zone diameters were determined for all isolates using a 30-μg temocillin disc (Oxoid, UK). Initial screening for ESβL, AmpC, and carbapenemase

Tab. II. Bacterial profile of biofilms formed on tracheostomy tubes (TTs) collected from patients of the 5th Polish Military Hospital with Polyclinic in Krakow (Poland).

TTS ID	% (N)	TYPE OF CO-COLONIZATION BACTERIAL SPECIES
	100 (8)	**Monomicrobial (1 species)**
TT_27.28.34	37.5 (3)	*S. aureus*
TT_6.7	25 (2)	*E. coli*
TT_20	12.5 (1)	*S. marcescens*
TT_33	12.5 (1)	*E. cloaceae*
TT_39	12.5 (1)	*K. pneumoniae*
	100 (11)	**Bimicrobial (2 species)**
TT_3.24	18.2 (2)	*S. aureus* + *P. aeruginosa*
TT_1	9.1 (1)	*P. acnes* + *C. albicans*
TT_5	9.1 (1)	*C. macginleyi* + *S. epidermidis*
TT_10	9.1 (1)	*S. aureus* + *E. cloaceae*
TT_25	9.1 (1)	*S. aureus* + *C. albicans*
TT_29	9.1 (1)	*S. aureus* + *S. epidermidis*
TT_30	9.1 (1)	*Corynebacterium spp.* + *C. albicans*
TT_36	9.1 (1)	*E. faecalis* + *K. pneumoniae*
TT_37	9.1 (1)	*S. aureus* + *S. maltophilia*
TT_45	9.1 (1)	*S. viridans* + *C. albicans*
	100 (14)	**Polymicrobial (3 species)**
TT_4	7.1 (1)	*C. macginleyi* + *S. aureus* + *P. aeruginosa*
TT_9	7.1 (1)	*S. epidermidis* + *S. salivarius* + *C. albicans*
TT_12	7.1 (1)	*C. macginleyi* + *S. epidermidis* + *P. acnes*
TT_13	7.1 (1)	*Corynebacterium spp.* + *epidermidis* + *S. viridans*
TT_14	7.1 (1)	*S. agalactiae* + *S. viridans* + *M. catarrhalis*
TT_16	7.1 (1)	*S. aureus* + *E. cloaceae* + *K. pneumoniae*
TT_17	7.1 (1)	*S. epidermidis* + *S. viridans* + *E. coli*
TT_18	7.1 (1)	*S. epidermidis* + *S. viridans* + *Neisseria spp.*
TT_26	7.1 (1)	*S. aureus* + *E. coli* + *C. albicans*
TT_31	7.1 (1)	*S. agalactiae* + *E. coli* + *K. pneumoniae*
TT_35	7.1 (1)	*S. aureus* + *S. epidermidis* + *Neisseria spp.*
TT_41	7.1 (1)	*S. aureus* + *K. pneumoniae* + *C. albicans*
TT_42	7.1 (1)	*S. aureus* + *S. viridans* + *S. liquefaciens*
TT_43	7.1 (1)	*Proteus spp.* + *P. aeruginosa* + *C. albicans*
	100 (6)	**Polymicrobial (4 species)**
TT_11	16.7 (1)	*E. cloaceae* + *K. pneumoniae* + *Proteus spp.* + *S. maltophilia*
TT_21	16.7 (1)	*S. aureus* + *S. epidermidis* + *S. viridans* + *K. pneumoniae*
TT_32	16.7 (1)	*S. aureus* + *S. epidermidis* + *S. haemolyticus* + *Micrococcus spp.*
TT_38	16.7 (1)	*S. aureus* + *K. pneumoniae* + *S. liquefaciens* + *C. albicans*

Tab. II. cd. Bacterial profile of biofilms formed on tracheostomy tubes (TTs) collected from patients of the 5th Polish Military Hospital with Polyclinic in Krakow (Poland).

TTS ID	% (N)	TYPE OF CO-COLONIZATION BACTERIAL SPECIE
TT_40	16.7 (1)	*S. epidermidis* + *S. viridans* + *E. cloaceae* + *K. pneumoniae*
TT_44	16.7 (1)	*A. baumanii* + *K. pneumoniae* + *Proteus spp.* + *C.albicans*
	100 (4)	**Polymicrobial (5 species)**
TT_8	25 (1)	*E. faecalis* + *E. coli* + *K. pneumoniae* + *S. maltophilia* + *C. albicans*
TT_15	25 (1)	*S. epidermidis* + *S. viridans* + *Proteus spp.* + *P. aeruginosa* + *C. albicans*
TT_22	25 (1)	*Corynebacterium spp.* + *S. aureus* + *E. coli* + *E. cloaceae* + *K. pneumoniae*
TT_23	25 (1)	*S. aureus* + *E. cloaceae* + *K. pneumoniae* + *P. aeruginosa* + *S. maltophilia*
	100 (2)	**Polymicrobial (6 species)**
TT_2	50 (1)	*S. sciuri* + *K. pneumoniae* + *P. aeruginosa* + *C. albicans* + *C. parapsilosis* + *Sacharomyces spp.*
TT_19	50 (1)	*S. aureus* + *S. epidermidis* + *S. viridans* + *C. albicans* + *C. krusei* + *Sacharomyces spp.*

Abbreviations: TTs – tracheostomy tubes.

production was performed on the basis of the disc diffusion method using ceftazidime (30 µg), cefotaxime (30 µg), ceftriaxone (30 µg), cefoxitin (30 µg), imipenem (10 µg), meropenem (10 µg) and ertapenem (10 µg) discs (Liofilchem® MIC Test Strips) according to the EUCAST v. 9.0, 2019, screening criteria for β-lactamase production.

- **Scanning Electron Microscopy (SEM) observations.** The investigated TT biomaterials were characterized after microbiological analysis by the field-emission scanning electron microscope (FE-SEM, Hitachi S-4700). Bacteria in biofilms colonizing the inner surfaces of the tested TTs were fixed for the SEM observations following the protocols described elsewhere [18]. The tested polyvinyl chloride (PVC) TTs obtained from patients were cut into 2-mm-thick discs after 10 and 28 days of incubation. Briefly, TT samples were fixed in a 2.5% glutaraldehyde solution (Sigma-Aldrich, Germany) in 0.1 M Dulbecco's Phosphate Buffered Saline (pH = 7.4) (DPBS, Lonza) for 4 hours, and then rinsed twice for 10 minutes with DPBS. Afterwards, TT samples were dehydrated by passing them through the following ethanol series (50%, 60%, 70%, 80%, 96% and 100% ethanol) for 10 minutes each. The prepared samples were attached to the SEM holder using an adhesive carbon tape and coated with gold particles (via sputter coating with a ~15-nm layer of gold, Quorum Q150T S). Finally, image analyses were started.

RESULTS

General findings

Out of the total of 45 TTs studied, 100% were found to be positive in bacterial culture. From the TA samples collected during the study period, 58 PPMs (10 species) were isolated and identified. Most of the clinical materials were from the ORL ward (77.8%, n = 35) and the remaining materials were obtained from patients of the ICU ward (22.2%, n = 10). Thirty-seven TTs (82%) were inhabited by 2 or more microorganisms (Tab. II.).

Antibiotic resistance

- **Gram-positive PPMs.** Among Gram-positive PPMs, we found 16 isolates of *S. aureus* species (identified in 35.5% of the patients). In the case of the *S. aureus* strains examined, no MRSA isolates were detected. Three isolates (14.3%) were resistant to each individual fluoroquinolone (ciprofloxacin, levofloxacin, moxifloxacin and ofloxacin). Two strains (9.5%) were resistant to erythromycin and 2 (9.5%) to clindamycin. The phenotypic mechanism of MLSB resistance was demonstrated for 4 (25%) *S. aureus*, among which both mechanisms (cMLS$_B$ and iMLS$_B$) were detected in 2 (9.5%) isolates. In addition, 3 (14.3%) strains were resistant to tetracycline and trimethoprim-sulfamethoxazole (Tab. III.).
- **Gram-negative PPMs.** In the cases of Gram-negative PPMs, we isolated a total of 9 different species (42 isolates), wherein up to 76.2% (32 isolates) of the bacteria identified are representatives of the order *Enterobacterales* (family *Enterobacteriaceae*), followed by non-fermenting Gram-negative bacteria (NFGNB) including *P. aeruginosa* (n = 5, identified in 11.9%), *Stenotrophomonas maltophilia* (n = 4, in 9.5% of patients) and *Acinetobacter baumannii* (n = 1, in 2.4%), which constituted the remaining 23.8% of the studied isolates (Tab. III.). Among *Enterobacterales*, *Klebsiella pneumoniae* was the most frequently detected bacterium (n = 10, identified in 23.8% of the patients) followed by *Enterobacter cloacae* (n = 7, in 16.7% of patients); *Escherichia coli* was also frequent (n = 7, in 16.7% of patients), and subsequently *Proteus* spp. (n = 4, in 9.5% of patients); *Serratia marcescens* (n = 2, in 4.8% of patients) and *Serratia liquefaciens* (n = 2, in 4.8% of patients). Among the *K. pneumoniae* rods, 2 (20%) isolates were ESβL producers. Generally, *K. pneumoniae* isolates were highly resistant to penicillins and cephalosporins: 100% (n = 10) were resistant to ampicillin with sulbactam and ticarcillin with clavulanic acid; 90% (n = 9) to amoxicillin with clavulanic acid; 80% (n = 8) to ceftazidime; 70% (n = 7) to ceftriaxone and 20% (n = 2) to piperacillin and cefepime, respectively. In addition, 40% (n = 4) of *K. pneumoniae* were resistant to ciprofloxacin; 30% (n = 3) to levofloxacin and ofloxacin; 20% (n = 2) to moxifloxacin. In

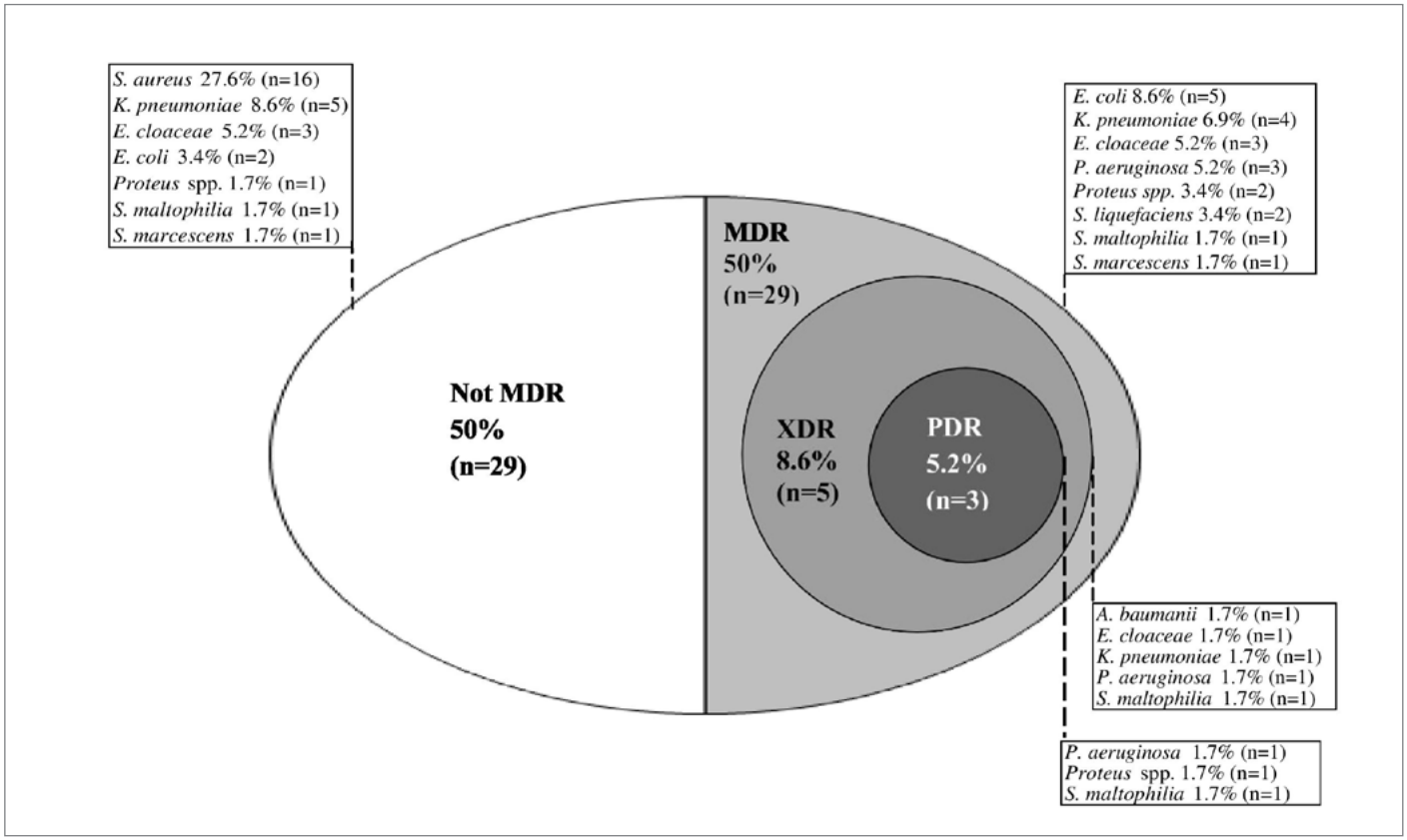

Fig.1. ??

the group of aminoglycosides, 40% (n = 4) of isolates were resistant to amikacin, 20% (n = 2) to gentamicin and tobramycin, and 1 (10%) netilmicin-resistant isolate was also found. Whereas 20% (n = 2) of strains were resistant to aztreonam and 10% (n = 1) were resistant to trimethoprim with sulfamethoxazole. Among the detected isolates of *E. cloacae*, 100% (n = 7) of strains were resistant to piperacillin and ticarcillin with clavulanic acid; 85.7% (n = 6) to ceftazidime and ceftriaxone; 42.6% (n = 3) to cefotaxime, cefepime, aztreonam and ciprofloxacin; 28.6% (n = 2) to moxifloxacin, ofloxacin, netilmicin and tobramycin; three isolates were also found to be resistant to different agents (each 14.3%): doripenem, ertapenem and levofloxacin. Among the strains of *E. coli*, all isolates (100%, n = 7) were resistant to ampicillin, ampicillin with sulbactam, amoxicillin, and ticarcillin with clavulanic acid; 85.7% (n = 6) to amoxicillin with clavulanic acid, 28.6% (n = 2) to piperacillin and levofloxacin, 14.3% (n = 1) to ceftazidime, ceftriaxone, ciprofloxacin and ofloxacin. In the cases of 4 strains of *Proteus* spp., 100% were resistant to all penicillins; 75% (n = 3) were resistant to ceftazidime and all analyzed fluoroquinolones and trimethoprim with sulfamethoxazole; 50% (n = 2) to cefotaxime, ceftriaxone, cefepime, doripenem, ertapenem, gentamicin and tobramycin; 25% (n = 1) to imipenem, meropenem, aztreonam, amikacin and netilmicin. Among the 2 strains of *S. liquefaciens*, 1 isolate (50%) was resistant to penicillin antibiotics, cephalosporins, fluoroquinolones, tobramycin and trimethoprim with sulfamethoxazole (Tab. III.). Among the 2 strains of *S. marcescens*, 100% of isolates were resistant to ampicillin with sulbactam, ticarcillin with clavulanic acid, ceftazidime and ceftriaxone; 50% (n = 1) were resistant to piperacillin, cefotaxime, aztreonam, moxifloxacin, ofloxacin, netilmicin and tobramycin. The most frequently identified pathogenic NFGNBs were *P. aeruginosa* followed by *S. maltophilia* and *A. baumannii*. The obtained results showed, among the isolated *P. aeruginosa*, the highest percentage (100%, n = 5) of strains resistant to piperacillin and cefepime; 80% (n = 4) of isolates were resistant to netilmicin; 60% (n = 3) were resistant to ceftazidime, ciprofloxacin and levofloxacin; 40% (n = 2) were resistant to doripenem, imipenem, meropenem, aztreonam and tobramycin. Among the 4 isolates of *S. maltophilia*, 100% were resistant to ticarcillin with clavulanic acid; 1 strain (25%) resistant to ciprofloxacin and 1 strain (25%) resistant to trimethoprim with sulfamethoxazole were reported. To sum up, the less frequently detected bacterium was *A. baumannii* and, moreover, this isolate was resistant to all antibiotics used, except tobramycin. Tab. III. presents the compilations of the most frequently detected Gram-positive and Gram-negative PPMs and associated antibiotic resistance. According to the criteria by Magiorakos et al., 29 MDR (50%) isolates were detected, including 5 XDR (8.6%) represented by different species and 3 strains (*P. aeruginosa*, *Proteus* spp., *S. maltophilia*) qualified to the PDR category, which accounted for 5.2% of the total pool of antibiotic-resistant pathogens (Fig. 1.).

Biofilm observation by SEM

SEM observations of the biofilms formed on the surface of the TT samples revealed a consistent and sustainable network of cellular

Tab. III. Most frequently detected Gram-positive and Gram-negative PPMs and related antibiotic resistance.

	STAPHYLOCOCCUS SPP.	ENTEROBACTERALES						NON-FERMENTING GRAM-NEGATIVE BACTERIA (NFGNB)		
Antibiotics	*S. aureus* n = 16	*K. pneumoniae* n = 10	*E. cloaceae* n = 7	*E. coli* n = 7	*Proteus* spp. n = 4	*S. liquefaciens* n = 2	*S. marcescens* n = 2	*P. aeruginosa* n = 5	*S. maltophilia* n = 4	*A. baumanii* n = 1
	Number of resistant isolates (%)									
Penicillins										
Ampicillin		ON	ON	7 (100)	4 (100)	ON	ON	ON	ON	ON
Ampicillin-sulbactam		10 (100)	0 (0)	7 (100)	4 (100)	1 (50)	2 (100)	ON	ON	1 (100)
Amoxicillin		ON	ON	7 (100)	4 (100)	ON	ON	ON	ON	ON
Amoxicillin-clavulanic acid		9 (90)	ON	6 (85.7)	4 (100)	ON	ON			ON
Piperacillin		2 (20)	7 (100)	2 (28.6)	4 (100)	1 (50)	1 (50)	5 (100)	ON	1 (100)
Ticarcillin-clavulanic acid		10 (100)	7 (100)	7 (100)	4 (100)	1 (50)	2 (100)		4 (100)	1 (100)
Cephalosporins								ON		
Ceftazidime		8 (80)	6 (85.7)	1 (14.3)	3 (75)	1 (50)	2 (100)	3 (60)	ON	1 (100)
Cefotaxime		1 (10)	3 (42.6)	0 (0)	2 (50)	0 (0)	1 (50)	ON	ON	ON
Ceftriaxone		7 (70)	6 (85.7)	1 (14.3)	2 (50)	1 (50)	2 (100)	ON	ON	ON
Cefepime		2 (20)	3 (42.6)	0 (0)	2 (50)	0 (0)	0 (0)	5 (100)	ON	1 (100)
Cefoxitin	0 (0)									
Carbapenems										
Doripenem		0 (0)	1 (14.3)	0 (0)	2 (50)	0 (0)	0 (0)	2 (40)	ON	1 (100)
Ertapenem		0 (0)	1 (14.3)	0 (0)	2 (50)	0 (0)	0 (0)	ON	ON	ON
Imipenem		0 (0)	0 (0)	0 (0)	1 (25)	0 (0)	0 (0)	2 (40)	ON	1 (100)
Meropenem		0 (0)	0 (0)	0 (0)	1 (25)	0 (0)	0 (0)	2 (40)	ON	1 (100)
Monobactams										
Aztreonam		1 (10)	3 (42.6)	0 (0)	1 (25)	0 (0)	1 (50)	2 (40)	ON	1 (100)
Fluoroquinolones										
Ciprofloxacin	3 (14.3)	4 (40)	3 (42.6)	1 (14.3)	3 (75)	0 (0)	0 (0)	3 (60)	1 (25)	1 (100)
Levofloxacin	3 (14.3)	3 (30)	1 (14.3)	2 (28.6)	3 (75)	1 (50)	0 (0)	3 (60)	ON	1 (100)
Moxifloxacin	3 (14.3)	2 (20)	2 (28.6)	0 (0)	3 (75)	1 (50)	1 (50)	0 (0)	ON	1 (100)
Ofloxacin	3 (14.3)	3 (30)	2 (28.6)	1 (14.3)	3 (75)	1 (50)	1 (50)	0 (0)	ON	1 (100)
Aminoglycosides										
Amikacin	0 (0)	4 (40)	0 (0)	0 (0)	1 (25)	0 (0)	0 (0)	0 (0)	ON	1 (100)
Gentamycin	0 (0)	2 (20)	0 (0)	0 (0)	2 (50)	0 (0)	0 (0)	0 (0)	ON	ON
Netylmycin	0 (0)	2 (20)	2 (28.6)	0 (0)	1 (25)	0 (0)	1 (50)	4 (80)	ON	1 (100)
Tobramycin	0 (0)	1 (10)	2 (28.6)	0 (0)	2 (50)	1 (50)	1 (50)	2 (40)	ON	1 (100)
Tetracyclines										
Tetracycline	3 (14.3)									
Oxazolidinones										

Tab. III. cd. Most frequently detected Gram-positive and Gram-negative PPMs and related antibiotic resistance.

	STAPHYLOCOCCUS SPP.	ENTEROBACTERALES						NON-FERMENTING GRAM-NEGATIVE BACTERIA (NFGNB)		
Antibiotics	*S. aureus* n = 16	*K. pneumoniae* n = 10	*E. cloaceae* n = 7	*E. coli* n = 7	*Proteus* spp. n = 4	*S. liquefaciens* n = 2	*S. marcescens* n = 2	*P. aeruginosa* n = 5	*S. maltophilia* n = 4	*A. baumanii* n = 1
Linezolid	0 (0)									
Macrolides, Lincosamides and Streptogramins										
Erythromycin	4 (25)									
Clindamycin	2 (9,5)									
$iMLS_B$	2 (9,5)									
$cMLS_B$	2 (9,5)									
Miscellaneous agents								ON		
Chloramphenicol	9 (56,2)									
Rifampicin	0 (0)									
Trimethoprim-sulfamethoxazole	3 (14,3)	1 (10)	1 (14,3)	0 (0)	3 (75)	0 (0)	1 (100)		1 (25)	1 (100)

Abbreviations: NFGNB – non-fermenting Gram-negative bacteria, NR – natural resistance, PPMs – potentially pathogenic microorganisms.

multilayers, being compositions from different bacterial species (Fig. 2A.–D.), surrounded and encircled by a protective biofilm matrix. The presented SEM images illustrate a typical bacterial biofilm, a complex multicellular structure of microorganisms encompassed by a layer of organic and inorganic substances produced by these organisms and showing adhesion to the abiotic surface, i.e. the biomaterial of the TTs. SEM images show that the identified isolated bacterial species gladly developed compatible and strong associations with other species and formed agglomerates on the TT surfaces. Toughened biofilm that is difficult to remove may pose a microbiological hazard.

DISCUSSION

The bacterial biofilm formed on the surface of the tracheostomy tube is a major problem in modern medicine [19]. It is now known that over 99% of bacteria in the natural environment are present in the form of biofilm. The formation of biofilm by pathogens is considered to be the main factor of virulence, protecting against the targeted action of antimicrobials, the host's immune response mechanisms and unfavorable environmental conditions [19]. Biofilm can arise on the surface of living cells because its formation is a natural feature of all bacteria that make up the microflora of the skin and mucous membranes. Also, pathogenic bacteria entering the body in the form of plankton, after the initial stage of adhesion to the host cells, create a biofilm in the gate of infection [11–13]. In addition, biofilm-forming bacteria are also able to permanently and effectively colonize abiotic surfaces, including biomaterials more and more commonly used in medicine for the production of endotracheal tubes and tracheostomy tubes [11–13]. The dynamic development in the field of biomaterials significantly contributed to the improvement of the quality of life of patients, but at the same time, it became the cause of an increased risk of developing infections associated with biofilm (Biomaterial-Associated Infections; BAIs) [12–14, 20–22]. It is currently shown that BAIs are responsible for approximately 65–80% of all infections in humans and animals. Similarly, according to the literature, the frequency of bacterial colonization in intubated and mechanically ventilated people may reach 80% [10].

Patients with tracheostomy constitute a special group of people constantly colonized with a diverse bacterial flora, most often environmental, with the dominant role of *S. aureus*, *P. aeruginosa* and *Acinetobacter* species [20, 23–25]. Several different species of Gram-negative and Gram-positive bacteria, including anaerobes and fungi, are most often isolated from tracheal aspirates from these patients, which makes it difficult to determine their role in infection [8–10, 20]. Almost all pathogenic organisms, including the above-mentioned ones and mycobacteria, have the ability to form biofilms on the surface of TTs and to cause BAIs [12–14, 21, 22]. It should be remembered that massive colonization of the tracheostomy tube along with improper care of the patient may lead to the displacement of microorganisms to the lower parts of the respiratory tract and initiation of the inflammatory process [7, 9, 10]. The need for mechanical ventilation is related to endotracheal intubation or tracheostomy and even when this invasive procedure is not combined with ventilation, there is a risk of infection due to the bypassing and impairment of the physiological pathway of this part of the respiratory system. Respiratory tract infections manifested by VAP (ventilator-associated pneumonia) are a serious group in terms of their frequency of occurrence [13].

The aim of this study was to learn about the bacteriological pattern of Gram-positive and Gram-negative PPMs colonizing tracheostomy tubes of civilian patients who underwent tracheotomy and were hospitalized in one of the hospitals in south-eastern Poland. The detailed objectives of the conducted analysis were the identification of the isolated Gram-positive and Gram-negative

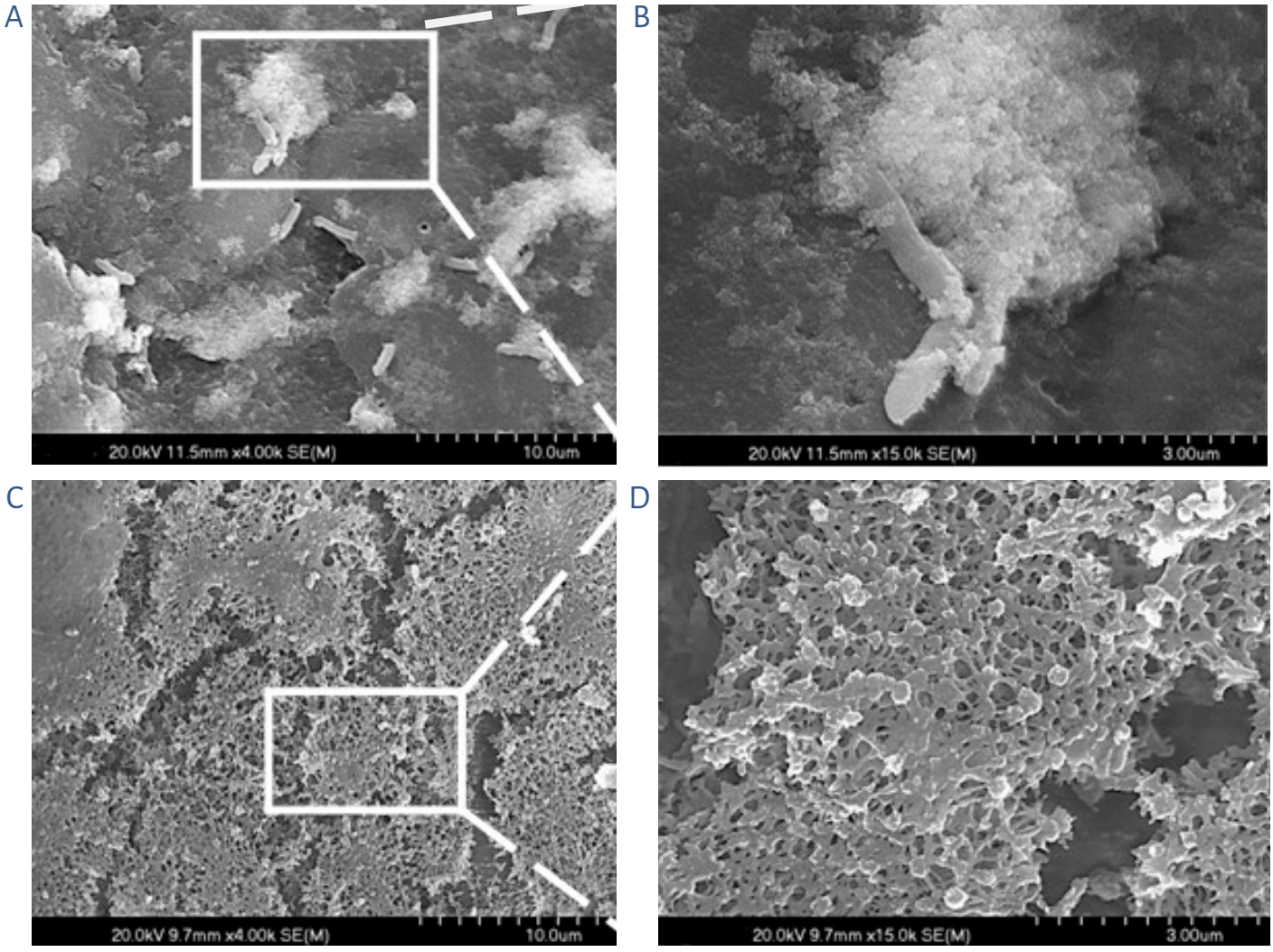

Fig. 2. (A-B) Low (A) and (B) high magnification SEM images of the bacterial biofilms formed on polyvinyl chloride (PVC) tracheostomy tubes obtained from patients 10 days after intubation; (C-D) low (C) and (D) high magnification SEM images of the bacterial biofilms formed on polyvinyl chloride (PVC) tracheostomy tubes obtained from patients 28 days after intubation.

PPMs and the determination of their antibiotic resistance profiles. Additionally, imaging of the resulting bacterial biofilm on the biomaterial of the tracheostomy tube was performed using scanning electron microscopy (SEM).

The present preliminary results constitute the starting point for further work on the modification of the surface of the tracheostomy tube biomaterial, which is aimed at reducing the risk of bacterial biofilm formation and delaying microbial colonization, implemented under the project entitled "Functionalization of the surface of polymeric biomaterials for dedicated implantation applications". In the analysis performed, all TTs collected (100%) were positive for the presence of microorganisms. The strains identified in the case of 9 tubes, in accordance with the assumptions adopted, were classified as non-PPMs due to their common occurrence in the environment and the fact that they constitute the physiological flora of the skin and mucous membranes. Therefore, they were treated as non-pathogenic and determination of their drug resistance profiles was abandoned [2, 9, 24]. It should be remembered that, as opportunistic microorganisms inhabiting the upper respiratory tract and forming biofilms on the biomaterials of the tracheostomy tube, they can cause a number of infections including corneal ulcer, urinary tract infection, intravenous catheter-related infection, valvular endocarditis and septicaemia in immunosuppressed patients [23, 24]. An example of such a microorganism may be the species *P. acne*s usually known as nonpathogenic and a part of the normal microbiota of the skin, oral cavity, gastrointestinal and genitourinary tracts [23]. This species of bacteria has been frequently reported to cause endogenous BAIs associated with the use of biomaterials employed in treatment, including tracheostomy tubes [23]. These infections are most often the result of colonization of the biomaterial at the time of its implantation or as a result of transient bacteremia [12].

In this study, rapid bacterial colonization of the TT biomaterial with the leading species, i.e. *S. aureus*, *K. pneumoniae* and *P. aeruginosa*, occurring within 1 day, was observed in 97.1% of patients of the ORL department. In 2021, research by Baidya et al. confirmed the bacterial colonization of TTs in 78.6% of mechanically ventilated patients with the leading culprits being *Pseudomonas* aeruginosa (31.0%) followed by *Acinetobacter calcoaceticus baumannii complex* (16.9%), *Klebsiella pneumoniae* (16.9%) [21].

In the above study, 72% of the most frequently isolated PPMs from tracheal aspirates were Gram-negative rods, while Gram-positive bacteria accounted for 28%. Similar results of such analyses were obtained by other authors. Namely, Patil et al. showed the dominance of Gram-negative organisms (80.6%) in relation to Gram-positive organisms (19.4%) [9], Khatun et al. Gram-negative 83%, Gram-positive 17% [14]. Overall, 58 PPMs of isolates (10 species) were identified with a predominance of *S. aureus* (35.5%) among Gram-positive bacteria and *K. pneumoniae* (23.8%) among Gram-negative bacteria. Among the Gram-negative PPMs, the most frequently isolated species were: *E. cloacae* (16.7%), *E. coli* (16.7%), *P. aeruginosa* (11.9%), *Proteus* spp. (9.5%), *S. maltophilia* (9.5%), *S. liquefaciens* (4.8%), *S. marcescens* (4.8%) and *A. baumannii* (2.4%). The results of a parallel study conducted by Shrestha et al. in 2021 showed the dominance of the following species among Gram-negative microorganisms colonizing ETTs in 188 patients of the ICU department: *Acinetobacter* spp. (51.82%), *K. pneumoniae* (19.7%), *P. aeruginosa* (16.78%), and *E. coli* (3.64%), while among Gram-positive microorganisms, it was *S. aureus* (4.37%) [25]. For comparison, in their publication, Scholte et al. presented the following data for individual species: *S. aureus* (8–19%), *K. pneumoniae* (7–12%), *P. aeruginosa* (17–33%), *Proteus* spp. (3-7%), and *S. maltophilia* (2–12%) [8]. In a study of bacterial biofilm on tracheostomy tubes by Raveendra et al., the following species were distinguished: *K. pneumoniae* (60%), *A. baumannii* (45%), *P. aeruginosa* (43.3%), *S. aureus* (28.3%), and *E. coli* (28.3%) [20].

Among the 45 tubes analyzed, 17.8% were monomicrobial tubes from which a single bacterial isolate was isolated, 24.4% were bimicrobial tubes with two species of bacteria, and from the remaining 57.8% of tubes three or more species of microorganisms were isolated. In the comparison proposed by Singhai et al., there were 83.3% of monomicrobial tubes and 16.7% of bimicrobial tubes, with polymicrobial tubes not tested [12].

We detected the presence of a high percentage of MDR (50%), XDR (8.6%) and PDR (5.2%) phenotypes in Gram-negative isolates. Singhai et al. (2012) confirmed a large amount (93.8%) of multidrug-resistant Gram-negative microorganisms causing DRI and a high percentage of ESβL producers (81.3%) in representatives of *K. pneumoniae* [12]. Contrarily, only 4.9% of ESβL-producing *K. pneumoniae* were detected in our study. The same research team noted a high percentage (60%) of multidrug-resistant *S. aureus* isolates [12]. In our study, no strains with the MDR phenotype were identified among the isolated *S. aureus*. Isolation of multidrug-resistant strains (MDR, XDR, PDR) from the tracheostomy tube is most often associated with prior hospitalization and nosocomial infection. In addition, the complex structure of the biofilm and the different physiological characteristics of the microorganisms that make it up partly explain their high resistance to various bactericidal agents, including resistance to antibiotics [8].

Novel, rational methods of reducing the amount of bacterial biofilm on the surface of tracheostomy tubes are still being looked for. It is very important to develop new biomaterials or to modify the already existing ones that are used for the production of TTs to increase their resistance to microbial adhesion, colonization and biofilm formation. The surface properties of biomaterials can be chemically modified by applying anti-adhesive coatings, changing the surface charge, structure, roughness, chemical activity, as well as hydrophilic and hydrophobic properties [26]. A promising strategy used in the fight against infections associated with the use of biomaterials is the construction of coatings with bactericidal/bacteriostatic properties made of noble metals or other antimicrobial materials [27]. An alternative solution to combat bacterial biofilm is coating TTs with antibiotics, including gentamicin, tobramycin, cephalothin sodium, amoxicillin or vancomycin hydrochloride [28]. It should be remembered that the activity of a number of the available antimicrobial agents on biofilm is significantly limited compared to the activity of these substances on free-flowing cells of the same microbial strains. This is mainly due to the layered structure of the biofilm and the presence of a polysaccharide matrix, which makes it difficult for a number of antimicrobial compounds to penetrate into its structure. In addition, the change in gene expression and the metabolism of microbial cells located in the deeper layers of the biofilm structure lead to a reduced sensitivity to many antimicrobial substances.

Currently, despite continuous research, an ongoing challenge for medicine, microbiology and chemistry is the search for novelty technologies to limit the formation of bacterial biofilms on the surface of TTs or the modification of these surfaces in order to prevent this phenomenon. Scientific literature emphasizes that systemic administration of antibiotics does not prevent bacterial colonization of the treacheostomy tube. Hence, it is necessary to functionalize the tubes with e.g., targeted antibiotics, in order to act on microorganisms directly at the colonization site.

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Corresponding author: Łukasz Ścibik; Department of Molecular Medical Microbiology, Chair of Microbiology, Faculty of Medicine, Jagiellonian University Medical College; 18 Czysta Street, 31-121 Krakow, Poland; e-mail: lukas.scibik@gmail.com / l.scibik@5wszk.com.pl

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